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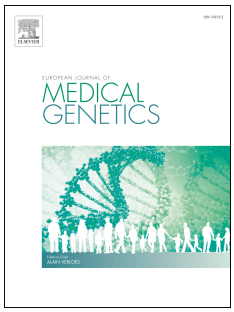

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Clinical research paperBenign Hereditary Chorea and deletions outside *NKX2-1:* what's the role of *MBIP?*

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Abstract

Heterozygous point mutations or deletions of the *NKX2-1* gene cause benign hereditary chorea (BHC) or a various combinations of primary hypothyroidism, respiratory distress and neurological disorders. Deletions proximal to, but not encompassing, *NKX2-1* have been described in few subjects with brain-lung-thyroid syndrome. We report on a three-generation Italian family, with 6 subjects presenting BHC and harboring a genomic deletion adjacent to *NKX2-1* and including the gene *MBIP*, recently proposed to be relevant for the pathogenesis of brain-lung-thyroid syndrome. We observed a clear reduction of *NKX2-1* transcript levels in fibroblasts from our patients compared to controls; this finding suggests that *MBIP* deletion affects *NKX2-1* expression, mimicking haploinsufficiency caused by classical *NKX2-1* related mutations.

Keywords: NKX2-1; MBIP; Benign Hereditary Chorea; brain-lung-thyroid syndrome

Introduction

Several *NKX2-1* point mutations or deletions of chromosome 14 encompassing *NKX2-1* have been associated with Benign Hereditary Chorea (BHC) [Breedveld et al. 2002; Thorwarth et al. 2014; Peall & Kurian 2015]. All patients were heterozygous for the mutation, therefore suggesting a dominant mode of inheritance. *NKX2-1* encodes a transcription factor, which is expressed during early development of thyroid, lung and forebrain regions. Accordingly, in addition to BHC, patients with *NKX2-1* mutations presented combinations of primary hypothyroidism, respiratory distress and neurological disorders. Few cases with BHC or brain-lung-thyroid syndrome have been reported to harbor deletions proximal to, but not disrupting, *NKX2-1* [Barnett et al. 2012;.Thorwarth et al. 2014; Kharbanda et al. 2017].

Here we describe a three-generation Italian family, with 6 subjects presenting with BHC and harboring a genomic deletion adjacent to *NKX2-1* and including the gene *MBIP*, recently proposed to be relevant for the pathogenesis of brain-lung-thyroid syndrome [Kharbanda et al. 2017].

Patients' Data

The probands were two siblings (III-1 and III-2, pedigree in Figure 1) with a very similar clinical picture (Table 1) consisting of hypotonia and motor delay, a hyperkinetic movement disorder noted since infancy and recurrent pulmonary infections. They both presented dysmorphic features (macrocephaly, prominent and large forehead, big round eyes, bilateral hallux valgus), oculomotor apraxia and generalized chorea with superimposed irregular myoclonic jerks. Cognitive functions were within normal limits. A younger half-sister (III-3) was referred for motor delay and unbalanced gait with frequent falls at age 4. The father (II-3) was affected by an early-onset, non progressive chorea and pulmonary pathology (severe asthma); after age 30, he suffered episodes of hypotension and hypoglycaemia. His brother (II-5) presented mild chorea associated with minor cognitive and psychiatric involvement; at age 35 he was diagnosed with lung carcinoma. Their grandmother (I-1) had only a mild early-onset chorea and she did not experience any pulmonary disease.

Thyroid function was measured in all cases during the follow-up and always resulted normal. The movement disorder spontaneously improved over time in all cases, causing little or no functional disability.

Methods

Informed consent for the genetic diagnosis was obtained from patients or parents according to the local regulations, DNA was extracted from venous peripheral blood lymphocytes according to standard procedures and was used as a template to amplify the 3 exons of the *NKX2-1* gene. PCR

fragments were analyzed by automated nucleotide sequencing using the Big-Dye terminator Ready Reaction Kit version 2 on a 3100 Genetic Analyzer Automated Sequencer (Applied Biosystems). Haplotype analysis was performed by SNPs array (ILLUMINA HumanCytoSNP-12 BeadChip). For whole exome sequencing (WES), genomic DNA was processed for library enrichment according to the SeqCap EZ Exome v3.0 kit (Roche). Sequencing was performed on an Illumina® Genome Analyzer HiSeq2500. The sequencing reads were aligned to the National Center for Biotechnology Information human reference genome (GRCh37/hg19) using the Burrows-Wheeler Aligner (BWA). Variant calling was performed using Genome Analysis Toolkit (GATK) and VariantStudio Data Analysis Software (Illumina®) was used for variants annotation and filtering. Coverage analysis was performed on WES data of individuals I-1 and III-2 using CANOES (http://www.columbia.edu/~ys2411/canoes). CANOES is a bioinformatics tool based on an algorithm for the detection of rare copy number variants from exome sequencing data; it uses a regression-based approach on selected reference samples in a given dataset to estimates variance of the read counts for an affected sample.

For gene content analysis, real-time quantitative PCR (RT-qPCR) was performed using SYBR Green chemistry and primers specific to *MBIP* exon 1 and 8, with a single copy gene (RNaseP) taken as a nuclear gene reference. For the analysis of transcripts, total RNA was extracted from fibroblasts patient, according to the manufacturer's instructions (RNeasy Mini Kit Qiagen). RT-qPCR assay on cDNA (Go Taq^{R} 2 step RTqPCR System) was performed using three different primers specific for amplification of *NKX2-1* and and one house-keeping gene (*ACTB*).

Results

Because of the clinical presentation, we sequenced *NKX2-1* in the proband III-1 but no mutation was found. In order to identify the genetic defect, we performed SNP-array analysis and WES. SNP-array analysis on available DNAs from the family revealed a genomic deletion on chr14:36206651-36321589 [GRCh38/hg38] (LOVD Variant ID: 0000352599) in 5 affected family members, absent in two unaffected cases (Figure 1). The identified deletion was near *NKX2-1*, but not encompassing this gene. Only one gene, *MBIP* (encoding MAP3K12 binding inhibitory protein 1), was included in the deleted region (Figure 1). WES was performed on individuals I-1 and III-2. Filtering steps using chorea genes (*NKX2-1, SGCE, SLC16A2, ADCY5*) as a bait, did not identified any candidate variants shared by the two affected cases. Because of the negative results from WES and the published evidence that copy number changes in next proximity to *NKX2-1* may cause a phenotype comparable to that of intragenic mutations [Kharbanda et al. 2017; Thorwarth et al. 2014], we pointed our attention to the chr14q13 deletion. We demonstrated the heterozygous deletion of *MBIP* in all affected members of the family (Figure 1) by quantitative PCR. Moreover, a

deep analysis of WES coverage data further confirmed this finding, highlighting that only *MBIP* has a halved copy number whereas the surrounding genes, including *NKX2-1*, were not affected (Supplementary Figure S1). In order to evaluate any possible effect of the *MBIP* deletion on *NKX2-1* expression, we measured *NKX2-1* transcript levels in patients II-3, III-1 and III-2. Despite the low expression of *NKX2-1* in fibroblasts, we observed a clear reduction of *NKX2-1* transcript levels in our patients compared to controls, although slightly lower than the decrease observed in fibroblasts from two patients harboring mutations in *NKX2-1* (Supplementary Figure S2).

Discussion

The deletion we identified comprises a single gene, *MBIP*, not associated with any human disease. Notably, deletion of *MBIP* was present in all reported cases of 14q13 deletion adjacent to but not directly involving *NKX2-1* [Barnett et al. 2012;.Thorwarth et al. 2014; Kharbanda et al. 2017] (Figure 1).

All these data suggest two possible, non-exclusive, mechanisms: 1) MBIP impairment is directly related to the pathogenesis of BHC and other brain-lung-thyroid syndromes; 2) *MBIP* deletion affects genomic elements important for *NKX2-1* expression, resulting in NKX2-1 impairment, as for the classical BHC cases.

Data in support of both hypotheses have been published. An identical pattern of temporal and spatial expression for *MBIP* and *NKX2*-1, including basal ganglia, forebrain, thyroid and lung, was reported [Thorwarth et al. 2014]; moreover, the chromosomal region encompassing the two genes is part of one of the largest synteny blocks in humans [Santagati et al. 2003]. These findings may indicate that both genes are important for the same biological process, i.e. the proper development and function of brain, lung and thyroid. Nevertheless, the known roles of MBIP protein seem not directly related or coincident to NKX2-1 and its expression regulation of thyroid-specific genes and genes involved in morphogenesis. Lastly, no point mutations in *MBIP* were found in 74 BHC patients, previously negative for *NKX2-1* mutations [Thorwarth et al. 2014] or have ever been described, despite the wide and growing use of WES in several genetic diseases, including movement disorders [Singleton 2012], arguing against a direct role of MBIP dysfunction in BHC. Conversely, our data from patients' fibroblasts provide an experimental support to the hypothesis that a secondary impairment of *NKX2-1,* due to a deletion of the adjacent genomic region comprehending *MBIP,* mimics *NKX2-1* haploinsufficiency and can explain the overlapping phenotype between deletions outside of *NKX2-1* and intragenic *NKX2-1* mutations.

Predicted enhancer elements for *NKX2-1* have been reported in the deleted region of previous cases with BHC and without a direct involvement *NKX2-1*, suggesting that these elements could contribute to the observed phenotype and the impairment of *NKX2-1* transcription we observed in

cells from our patients [Kharbanda et al. 2017]. Another possible explanation is that these deletions hit topologically associating domains (TADs), blocks of chromatin which are fundamental, not only for genome organization but also for genomic function [Dixon et al. 2016]. It has been shown that TADs play important roles in modulating transcription [Gorkin et al. 2014] and their disruption may result in disease [Lupianez et al. 2016]. The genomic region including *NKX2-1* and *MBIP* may be part of a TAD and deletion of portion of this domain could alter the expression of all or part of the contained genes.

In conclusion, our data indicate that deletion of the *MBIP* gene is associated with benign hereditary chorea, possibly lowering *NKX2-1* expression and thus mimicking haploinsufficiency caused by *NKX2-1* mutations.

The authors declare no conflict of interest.

Acknowledgments

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Figure legend

Figure 1: Pedigree of the investigated family and genetic analyses

A. SNP-array analysis, performed on all available DNAs from the family (8 subjects: 6 affected and 2 healthy cases), revealed a genomic heterozygous deletion on chr14q13 in 5 affected family members, absent in two unaffected cases. The poor quality of the DNA from individual I-1 led to its exclusion from the analysis. Real-time quantitative PCR showed the heterozygous deletion of *MBIP* on genomic DNAs in all affected members of the family. Labels indicate the presence (del) or absence (no_del) of the 14q13 deletion by SNP-array analysis (in dark blue) or the *MBIP* deletion by RT-qPCR (in red).

B. Map of the Chr.14 genomic region surrounding *MBIP* and *NKX2-1* genes, with the deletions identified in our family and in previously published cases.

Table 1. Clinical features of the affected family members

Patient	Neurological	Respiratory	Thyroid	Psychiatric	Other features
III-1	Hypotonia, generalized chorea, myoclonic jerks	Recurrent pulmonary infections	/	Mild cognitive impairment	Dysmorphisms
III-2	Hypotonia, generalized chorea, myoclonic jerks, ataxia	Recurrent pulmonary infections	/	/	Dysmorphisms, macrocrania
III-3	Motor delay, unbalanced gait	/	/	/	/
II-3	Chorea	Severe asthma	/	/	Hypotension, hypoglycaemia
II-5	Mild chorea	Lung carcinoma	/	Minor cognitive and psychiatric involvement	/
I-1	Mild chorea	/	/	/	/

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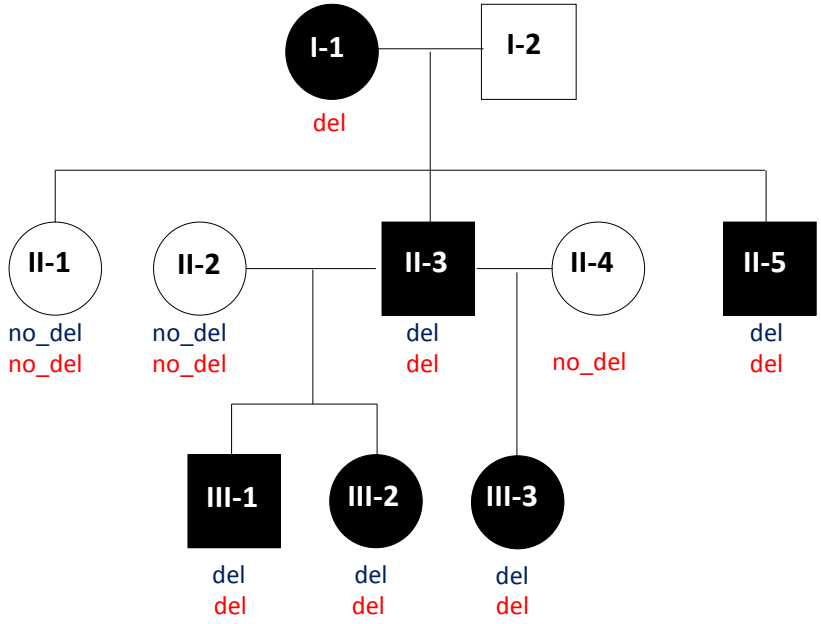
Supplementary Material includes 2 figures and is available online.

Supplementary Figure S1: Analysis of the coverage data from whole exome sequencing

Supplementary Figure S2: *NKX2-1* transcript analysis

A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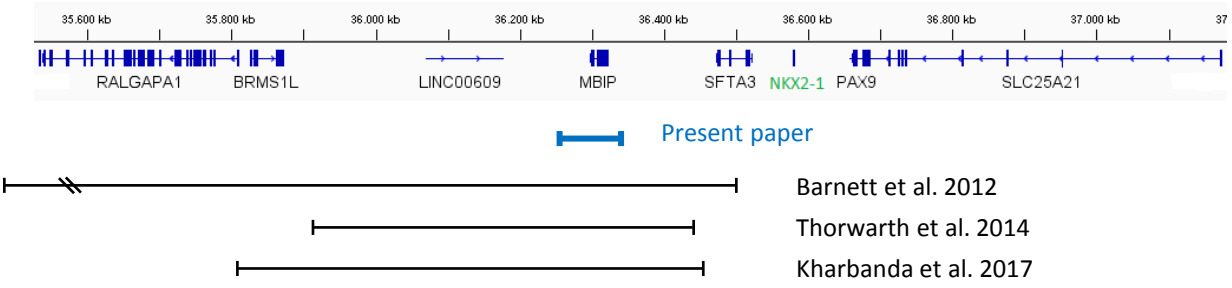
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Present paper
Barnett et al. 2012
Thorwarth et al. 2014
Kharbanda et al. 2017